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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 10/590,896 | 08/28/2006 | Toshihiro Ushijima | USHIJIMA3 | 5702 |

1444 7590 07/20/2011

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| EXAMINER | |
|---|---|
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| ART UNIT | PAPER NUMBER |
| 1645 | |
| MAIL DATE | DELIVERY MODE |
| 07/20/2011 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/590,896 | USHIJIMA ET AL. | |
| | Examiner | Art Unit | |
| | OLUWATOSIN OGUNBIYI | 1645 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *14 October 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-8,17 and 26-45* is/are pending in the application.

4a) Of the above claim(s) *1-7 and 26-45* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *8 and 17* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

***Continued Examination Under 37 CFR 1.114***

1 A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 7/14/10 has been entered.

2. A non-final Office Action was mailed on 8/18/10 in response to the RCE. However, the Office Action mailed 8/18/10 was vacated on 8/23/10 because a suspension of action was filed with the request for continued examination on 7/14/10.

In the interest of expediting prosecution, Applicants have filed an amendment on 10/14/10 addressing the rejections raised in the Office Action mailed 8/18/10.

Accordingly, the amendment filed 10/14/10 has been entered.

3. Claims 8 and 17 have been amended and are under examination Claims 9-16 and 18-25 have been cancelled. Claims 1-8, 17 and 26-45 are pending. Claims 1-7 and 26-45 are withdrawn further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 4/22/09.

***Claim Rejections Withdrawn***

4. The rejection of claims 14-16 and 23-25 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is withdrawn in view of the cancellation of the claims.

5. The rejection of claims 8, 14-17 and 23-25 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn in view of the amendments to the claims.

***Claim Rejections -Maintained***

***Claim Rejections - 35 USC § 112***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. The rejection of claims 8 and 17 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is maintained. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention is maintained. **This is a written description rejection.**

Independent claims 8 and 17 and dependent claims are drawn to:

an isolated variant of an *Erysipelothrix rhusiopathiae* surface protective antigen SpaA protein or

of a shortened form thereof (known as ΔSpaA protein), which is a shortened form of the SpaA protein in which a portion of the SpaA protein is deleted, wherein the SpaA protein has the amino acid sequence of SEQ ID NO: 2, and the ΔSpaA protein is a shortened form of the SpaA protein in which about one third of the C-terminal of the SpaA protein is deleted,

wherein said variant is immunogenic and expressed in E. coli as inclusion bodies and is selected from the group consisting of :

(1) the SpaA protein comprising the amino acid substitution at position 531 (arginine to glycine);

(2) the SpaA protein comprising the amino acid substitutions at position 214 (histidine to glutamine) and at position 253 (methionine to threonine);

(3) the ΔSpaA protein comprising the amino acid substitutions at position 214 (histidine to glutamine) and at position 253 (methionine to threonine);

(4) the ΔSpaA protein comprising the amino acid substitutions at position 69 (glutamic acid to glycine), at position 154 (glutamic acid to glycine), and at position 203

(isoleucine to threonine); and

(5) the ΔSpaA protein comprising the amino acid substitution at position 278 (aspartic acid to glycine).

The genus of the ΔSpaA protein as listed in 3-5, includes the ΔSpaA protein with the indicated amino acid substitutions and deletion of any 1/3$^{rd}$ of the C-terminal of the SpaA protein.

The claims require that the genus of ΔSpaA protein be immunogenic and form inclusion bodies when expressed in E. coli.

Actual Reduction to Practice

***SEQ ID NO: 2 is the full length amino acid sequence of the SpaA protein of Fujisawa strain. See p. 21 lines 5-11. SEQ ID NO: 2 did not form inclusion bodies in E. coli. See table 1 for the Fujisawa strain.***

As to a shortened form of SpaA known as ΔSpaA protein, the specification teaches cloning of this shortened form of SpaA from type 1 Fujisawa strain and Koganai strain and type 2 Tama 96 strain and SE9 strain wherein **the shortened from of SpaA is encoded by a partial SpaA gene with a nucleotide sequence of from 79$^{th}$ to 1260$^{th}$ nucleotide and codes for a shortened form of SpaA protein (with deletion of 207 amino acid residues at the C-terminal). See p. 30-31 example 1.**

The specification proceeds to determine whether inclusion bodies of SpaA and ΔSpaA protein form inclusion bodies and it was determined that out of all of the ΔSpaA from the

Fujisawa strain, Koganai strain, Tama 96 strain, SE9 strain only *E. coli* expressing ΔSpaA from the SE-9 strain formed inclusion bodies in *E. coli*. Also, for the full length SpaA protein, only *E. coli* expressing full length SpaA from the SE-9 strain was determined to form inclusion bodies in *E. coli*. See p. 34 table 1.

Table 1

| | Clones forming inclusion bodies/Clones expressing ΔSpaA | Clones forming inclusion bodies/Clones expressing SpaA |
|---|---|---|
| Fujisawa strain (type 1) | 0/3 | ND |
| SE-9 strain (type 2) | 3/30 | 1/15 |
| Tama 96 strain (type 2) | 0/3 | ND |
| Koganai strain (type 1) | 0/3 | ND |

For the four *E. coli* clones expressing SpaA and ΔSpaA of the SE 9 strain, plasmids were extracted and the amino acid substitutions were discovered as compared with wild type sequence of the SE9 strain (i.e. SEQ ID NO: 7) as set forth in table 2 p. 34.

Table 2

| Nucl. position | Nucleotide substitution (corresponding amino acid substitution) | Clone |
|---|---|---|
| 206th | A to G (the 69th glutamic acid to glycine) | No. 2 |
| 461st | A to G (the 154th glutamic acid to glycine) | No. 2 |
| 608th | T to C (the 203rd isoleucine to threonine) | No. 2 |
| 642nd | T to G (the 214th histidine to glutamine) | No. 1 |
| 758th | T to C (the 253rd methionine to threonine) | No. 1 |
| 833rd | A to G (the 278th aspartic acid to glycine) | No. 3 |
| 1591st | A to G (the 531st arginine to glycine) | No. 4 |

The discovered ability of the amino acid substitutions to form inclusion bodies, was confirmed by replacing portions of the SpaA and ΔSpaA of the SE 9 strain wild type sequence (SEQ ID NO: 7) with corresponding portions harboring the amino acid substitution and transforming *E.coli* to determine that inclusion bodies were formed. See example 2, p. 35-41.

Of the SE9 strain SpaA and ΔSpaA protein harboring amino acid substitutions wherein the SpaA and ΔSpaA protein substitution mutants formed inclusion bodies in *E. coli*, the specification reduced to practice the immunogenicity of the following in table 3:

Table 3

| Purified protein | ΔSpaA | | | SpaA | |
|---|---|---|---|---|---|
| Site of subst. in SpaA gene | 642nd 758th | 206th 461st 608th | 833rd | 1591st | 642nd 758th |
| Protein conc. (mg/ml) | 2.30 | 1.91 | 2.33 | 2.11 | 2.28 |
| Fold of dilution | No. of survival /No. of challenged | No. of survival /No. of challenged | No. of survival /No. of challenged | No. of survival /No. of challenged | No. of survival /No. of challenged |
| 625 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| 3125 | 9/10 | 8/10 | 10/10 | 10/10 | 10/10 |
| 15625 | 5/10 | 0/10 | 4/10 | 4/10 | 6/10 |
| 78125 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| Median protective dose in mice (ug) | 0.0864 | 0.1685 | 0.0875 | 0.0793 | 0.0621 |

The specification does not reduce to practice any variant of SEQ ID NO: 2 (SEQ ID NO: 2 is the full length amino acid sequence of the SpaA protein of Fujisawa strain. See p. 21 lines 5-11) including SEQ ID NO: 2 with the indicated amino acid substitutions and deletion of any 1/3$^{rd}$ of the C-terminal of the SpaA protein that forms inclusion bodies and remains immunogenic.

In fact the specification on table 1 p. 34 teaches that SEQ ID NO: 2 SpaA protein of Fujisawa strain with **deletion of 207 amino acid residues at the C-terminal encoded by a nucleotide sequence of from 79$^{th}$ to 1260$^{th}$ nucleotide of the corresponding SpaA gene,** see p. 30-31 example, did not form inclusion bodies which is required by the claims.

Sufficient Relevant Identifying Characteristics

The specification does not describe other members of the genus of proteins to which the claims are drawn that forms in inclusion bodies when expressed in *E. coli* and that is immunogenic.

Apart from the SpaA or ΔSpaA protein of the SE9 strain substitution mutants that are expressed as inclusion bodies in *E.coli* and are immunogenic as set forth in table 3, the specification does not describe the amino acid substitution(s) of any other type of SpaA protein including SEQ ID NO: 2 of Fujisawa strain and shortened form thereof with a 3rd of the C terminal deleted being expressed in *E. coli* as inclusion bodies and that are also immunogenic.

The specification does not disclose complete structure, partial structure, physical or chemical properties of other members of the genus to which the claims are drawn that form inclusion bodies and that are immunogenic.

The specification does not describe the structure common to all the members of the genus of SpaA or ΔSpaA protein variants that results in the function or characteristics of being expressed in *E. coli* as inclusion bodies and that are also immunogenic.

Method of making the claimed invention and Predictability in the art

While the specification teaches how to make SEQ ID NO: 2 SpaA protein of Fujisawa strain with **deletion of 207 amino acid residues at the C-terminal encoded by a nucleotide sequence of from 79th to 1260th nucleotide of the corresponding SpaA gene,** see p. 30-31 example, **the specification teaches that this did not form inclusion bodies and did not describe the claimed substitution mutants of SEQ ID NO: 2 that are immunogenic and form inclusion bodies in E. coli. See table 1**. As evidenced by table 1, it is unpredictable that other SpaA proteins or deletion variants of SpaA protein other than those of the SE9 strain can form inclusion bodies when expressed in *E. coli*, as evidenced by table 1.

Furthermore, as to the claimed and shortened form that comprise a deletion in any 1/3 of the c-terminal of SEQ ID NO; 2 and further comprise one or combination of the amino acid substitutions listed in the claims, the specification does not describe the common structure i.e. the immunoepitope(s) of said genus that correlates with function i.e. immunogenicity so that one of skill in the art can envision which 1/3rd of SEQ ID NO: 2 can be deleted in and still retain immunogenicity.

Colman et al. (Research in Immunology 145: 33-36, 1994, p.33 column 2, p. 35 column 1) disclose that a single amino acid change in an antigen can effectively abolish the interaction with an antibody. This underlies the importance of the description of the immunoepitope(s) and which amino acid deletion(s) and where and coupled with the instant amino acid substitutions in the deletion variant still retains immunogenicity. Houghten et al. (New Approaches to Immunization, Vaccines 86, Cold Spring Harbor Laboratory, p. 21-25, 1986) taught the criticality of individual amino acid residues and their positions in peptide antigen-antibody interactions. Houghten et al state (see page 24): "One could expect point mutations in the protein antigen to cause varying degrees of loss of protection, depending on the relative importance of the binding interaction of the altered residue. A protein having multiple antigenic sites, multiple point mutations, or accumulated point mutations at key residues could create a new antigen that is precipitously or progressively unrecognizable by any of the antibodies in the polyclonal pool."

The disclosure of variants of the SE9 strain of the SpaA protein that are immunogenic and form inclusion bodies does not provided written description support for the claimed variants of SEQ ID NO: 2 of the Fujisawa strain that form inclusion bodies and are immunogenic. **This is unpredictable because specification on table 1 p. 34 teaches that SEQ ID NO: 2 (Fujisawa strain SpaA) did not form inclusion bodies and SpaA protein of Fujisawa strain SEQ ID NO: 2 with deletion of 207 amino acid residues at the C-terminal encoded by a nucleotide sequence of from 79$^{th}$ to 1260$^{th}$ nucleotide of the corresponding SpaA gene,** see p. 30-31 example, did not form inclusion bodies.

In such an unpredictable art of protein mutation and the effect on antigenicity or immunogenicity as set forth supra, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus. In the instant case, the specification only discloses that the SpaA and ΔSpaA proteins of the SE 9 strain (SE9 strain's spa protein is SEQ ID NO: 7) as set forth in table 1 formed inclusion bodies in E.coli and with the particular amino acid substitutions were immunogenic. See *Noelle v Lederman*. 355 F. 3d 1343, 1350, 69 USPQ2d 1508, 1514 (*Fed. Cir. 2004*) and *In re Alonso* (Fed. Cir. 2008-1079). The specification has not reduced to practice any other SpaA and ΔSpaA

protein from any other strain of *E. rhusiopathiae* including the Fujisawa strain SEQ ID NO: 2 that that results in a protein that forms inclusion bodies in *E. coli* and is immunogenic.

In view of the above considerations, Applicants at the time of filing was not in possession of the genus claimed variants of SEQ ID NO: 2 including those comprising a deletion in about 1/3 of the C-terminal of SEQ ID NO: 2; and wherein the genus forms inclusion bodies when expressed in *E.coli* and are immunogenic.

***Applicants' arguments:***

Applicants state that claims 8 and 17 been amended to include the SEQ ID NO (SEQ ID NO: 2) in place of identification with a strain and to restrict the SpaA and ΔSpaA proteins to the five variant peptides which the Examiner (at page 8 of the Office Action) indicated are supported by the disclosure. In other words, the claims have been amended to the subject indicated by the Examiner has being supported
and enabled by the disclosure.

Applicants' argument is considered but is not persuasive. The previous office action of 8/18/10 (which was mailed out and later vacated) states on p. 8 that:

Therefore, the specification reduced to practice at the time of filing :

(1) The SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at position 531 i.e. 531st arginine to glycine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(2) The SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at both of position 214 i.e.214st histidine to glutamine and 253$^{rd}$ methionine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(3) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising both amino acid substitutions 214th histidine to glutamine and 253$^{rd}$ methionine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(4) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising all three amino acid substitutions comprising the 69$^{th}$ glutamic acid to glycine and 154$^{th}$ glutamic acid to glycine and 203$^{rd}$ isoleucine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(5) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising the amino acid substitution the 278$^{th}$ aspartic acid to glycine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies. See table 1, 2 and 3 of the specification.

According to the specification SE9 strain has the spaA protein set forth in SEQ ID NO: 7.

The subject matter supported by the specification as disclosed on p. 8 of the 8/18/10 action is drawn to the particular SpaA or ΔSpaA of SE9 strain whereas the claims are drawn to the SpaA of Fujisawa strain i.e. SEQ ID NO: 2. Applicants at the time of filing were not in possession of the genus claimed variants of SEQ ID NO: 2 including those comprising a deletion in about 1/3 of the C-terminal of SEQ ID NO: 2; and wherein the genus forms inclusion bodies when expressed in *E.coli* and are immunogenic for the reasons set forth in the above rejection.

***New Claim Objections/Rejections***

***Claim Objections***

7. Claims 8 and 17 are objected to because of the following informalities:

In claim 8 line 7, "in which a about one third" should be "in which about one third".

In claim 17 line 7, "in which a about one third" should be "in which about one third".

Appropriate correction is required.

***Claim Rejections - 35 USC § 112***

8. Claims 8 and 17 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains,

or with which it is most nearly connected, to make and/or use the invention. **This is an enablement rejection**.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention and Breadth of the Claims

Independent claims 8 and 17 and dependent claims are drawn to:

an isolated variant of an *Erysipelothrix rhusiopathiae* surface protective antigen SpaA protein or

of a shortened form thereof (known as ΔSpaA protein), which is a shortened form of the SpaA protein in which a portion of the SpaA protein is deleted, wherein the SpaA protein has the amino acid sequence of SEQ ID NO: 2, and the ΔSpaA protein is a shortened form of the SpaA protein in which a about one third of the C-terminal of the SpaA protein is deleted,

wherein said variant is immunogenic and expressed in E. coli as inclusion bodies and is selected from the group consisting of:

(i) the SpaA protein comprising the amino acid substitution at position 531 (arginine to glycine);

(2) the SpaA protein comprising the amino acid substitutions at position 214 (histidine to glutamine) and at position 253 (methionine to threonine);

(3) the ΔSpaA protein comprising the amino acid substitutions at position 214 (histidine to glutamine) and at position 253 (methionine to threonine);

(4) the ΔSpaA protein comprising the amino acid substitutions at position 69 (glutamic acid to glycine), at position 154 (glutamic acid to glycine), and at position 203 (isoleucine to threonine); and

(5) the ΔSpaA protein comprising the amino acid substitution at position 278 (aspartic acid to glycine).

The breadth of ΔSpaA protein as listed in 3-5, includes the ΔSpaA protein with the indicated amino acid substitutions and deletion of any 1/3$^{rd}$ of the C-terminal of the SpaA protein.

The claims require that SpaA protein variant and ΔSpaA protein be immunogenic and form inclusion bodies when expressed in E. coli.

Guidance in the Specification/The Existence or Absence of Working Examples

SEQ ID NO: 2 is the full length amino acid sequence of the SpaA protein of Fujisawa strain. See p. 21 lines 5-11.

As to a shortened form of SpaA known as ΔSpaA protein, the specification teaches cloning of this shortened form of SpaA from type 1 Fujisawa strain and Koganai strain and type 2 Tama 96 strain and SE9 strain wherein the shortened from of SpaA is encoded by a partial SpaA gene with a nucleotide sequence of from 79$^{th}$ to 1260th nucleotide and codes for a shortened form of SpaA protein **(with deletion of 207 amino acid residues at the C-terminal).** See p. 30-31 example 1.

The specification proceeds to determine whether inclusion bodies of SpaA and ΔSpaA protein from each of these strains form inclusion bodies and it was determined that out of all of the ΔSpaA from the Fujisawa strain, Koganai strain, Tama 96 strain, SE9 strain only *E. coli* expressing ΔSpaA from the SE-9 strain formed inclusion bodies in *E. coli*. Also, for the full length SpaA protein, only *E. coli* expressing full length SpaA from the SE-9 strain was determined to form inclusion bodies in *E. coli*. See p. 34 table 1.

***Therefore, the ΔSpaA from the SE-9 strain formed inclusion bodies (SE9 SpaA is SEQ ID NO: 7), but the ΔSpaA of SEQ ID NO: 2 (fujisawa strain) did not.***

Table 1

| | Clones forming inclusion bodies/Clones expressing ΔSpaA | Clones forming inclusion bodies/Clones expressing SpaA |
|---|---|---|
| Fujisawa strain (type 1) | 0/3 | ND |
| SE-9 strain (type 2) | 3/30 | 1/15 |
| Tama 96 strain (type 2) | 0/3 | ND |
| Koganai strain (type 1) | 0/3 | ND |

For the four *E. coli* clones expressing SpaA and ΔSpaA of the SE 9 strain, plasmids were extracted and the amino acid substitutions were discovered as compared with wild type sequence of the SE9 strain (i.e. SEQ ID NO: 7) as set forth in table 2 p. 34.

Table 2

| Nucl. position | Nucleotide substitution (corresponding amino acid substitution) | Clone |
|---|---|---|
| 206th | A to G (the 69th glutamic acid to glycine) | No. 2 |
| 461st | A to G (the 154th glutamic acid to glycine) | No. 2 |
| 608th | T to C (the 203rd isoleucine to threonine) | No. 2 |
| 642nd | T to G (the 214th histidine to glutamine) | No. 1 |
| 758th | T to C (the 253rd methionine to threonine) | No. 1 |
| 833rd | A to G (the 278th aspartic acid to glycine) | No. 3 |
| 1591st | A to G (the 531st arginine to glycine) | No. 4 |

The discovered ability of the amino acid substitutions to form inclusion bodies, was confirmed by replacing portions of the SpaA and ΔSpaA of the SE 9 strain wild type sequence with corresponding portions harboring the amino acid substitution and transforming *E. coli* to determine that inclusion bodies were formed. See example 2, p. 35-41.

Of the SE9 strain SpaA and ΔSpaA protein harboring amino acid substitutions wherein the SpaA and ΔSpaA protein substitution mutants formed inclusion bodies in *E. coli*, the specification reduced to practice the immunogenicity of the following in table 3:

Table 3

| Purified protein | ΔSpaA | | | SpaA | |
|---|---|---|---|---|---|
| Site of subst. in SpaA gene | 642nd 758th | 206th 461st 608th | 833rd | 1591st | 642nd 758th |
| Protein conc. (mg/ml) | 2.30 | 1.91 | 2.33 | 2.11 | 2.28 |
| Fold of dilution | No. of survival /No. of challenged | No. of survival /No. of challenged | No. of survival /No. of challenged | No. of survival /No. of challenged | No. of survival /No. of challenged |
| 625 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |
| 3125 | 9/10 | 8/10 | 10/10 | 10/10 | 10/10 |
| 15625 | 5/10 | 0/10 | 4/10 | 4/10 | 6/10 |
| 78125 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| Median protective dose in mice (ug) | 0.0864 | 0.1885 | 0.0875 | 0.0793 | 0.0621 |

The specification **does not teach** that SEQ ID NO: 2 including SEQ ID NO: 2 with the indicated amino acid substitutions and deletion of any 1/3$^{rd}$ of the C-terminal of the SpaA protein forms inclusion bodies and remains immunogenic. SEQ ID NO: 2 is the full length amino acid sequence of the SpaA protein of Fujisawa strain. See p. 21 lines 5-11.

In fact the specification on table 1 p. 34 teaches that SEQ ID NO: 2 SpaA protein of Fujisawa strain with **deletion of 207 amino acid residues at the C-terminal encoded by a nucleotide sequence of from 79$^{th}$ to 1260$^{th}$ nucleotide of the corresponding SpaA gene,** see p. 30-31 example, did not form inclusion bodies.

No amino acid substitutions were determined in SpaA (SEQ ID NO: 2) of the Fujisawa strain to confer ability to form inclusion bodies.

The specification does not correlate the amino acid substitutions in the claims and deletion in any 1/3rd of the c-terminal of SEQ ID NO: 2 with formation of inclusion bodies and immunogenicity.

Predictability of the Art and Amount of experimentation Necessary

While the specification teaches how to make SEQ ID NO: 2 protein of Fujisawa strain with **deletion of 207 amino acid residues at the C-terminal encoded by a nucleotide sequence of from 79$^{th}$ to 1260$^{th}$ nucleotide of the corresponding SpaA gene,** see p. 30-31 example, the specification teaches that this did not form inclusion bodies and SEQ ID NO: 2 also did not form inclusion bodies and it is unpredictable whether substitution mutants of SEQ ID NO: 2 or SEQ ID NO: 2 with deletion in any 1/3 of the C-terminal in addition to the listed substitutions in the claims will form inclusion bodies when expressed in E. coli and also be immunogenic.

The specification did not provide guidance as to the claimed substitution mutants of SEQ ID NO: 2 or the shortened form thereof that are immunogenic and form inclusion bodies in E. coli. See table 1. As evidenced by table 1, it is unpredictable that other SpaA proteins or deletion variants of SpaA protein other than those of the SE9 strain (which has the SpaA protein set forth in SEQ ID NO; 7) can form inclusion bodies when expressed in *E. coli*, as evidenced by table 1.

Furthermore, as to the claimed shortened form that comprise a deletion in any 1/3 of the c-terminal and further comprise one or combination of the amino acid substitutions listed in the claims, it is unpredictable whether these will still retain immunogenicity.

Colman et al. (Research in Immunology 145: 33-36, 1994, p.33 column 2, p. 35 column 1) disclose that a single amino acid change in an antigen can effectively abolish the interaction with an antibody. This underlies the importance of the description of the immunoepitope(s) and which amino acid deletion(s) and where and coupled with the instant amino acid substitutions in the deletion variant still retains immunogenicity. Houghten et al. (New Approaches to Immunization, Vaccines 86, Cold Spring Harbor Laboratory, p. 21-25, 1986) taught the criticality of individual amino acid residues and their positions in peptide antigen-antibody interactions. Houghten et al state (see page 24): "One could expect point mutations in the protein antigen to cause varying degrees of loss of protection, depending on the relative

importance of the binding interaction of the altered residue. A protein having multiple antigenic sites, multiple point mutations, or accumulated point mutations at key residues could create a new antigen that is precipitously or progressively unrecognizable by any of the antibodies in the polyclonal pool."

The disclosure of variants of the SE9 strain of the SpaA protein that are immunogenic and form inclusion bodies does not enable support for the claimed variants of SEQ ID NO: 2 of the Fujisawa strain that form inclusion bodies and are immunogenic because there is no disclosure as to how the same deletions and/or substitutions in the protein sequence of the SE9 strain that confer immunogenicity and formation of inclusion bodies of E. coli corresponds to the protein sequence of SpaA of the Fujisawa strain in terms of formation of inclusion bodies and immunogenicity and it is not clear whether the amino acid sequence of the SpaA protein of Fujisawa and the SE9 strain are the same or differ. Moreover, table 1 discloses that ΔSpaA of the SEQ ID NO: 2 did not form inclusion bodies (see table 1 Fujisawa strain).

In view of the above considerations, the specification is not enabled for the claimed variants of SEQ ID NO: 2 including those comprising a deletion in about 1/3 of the C-terminal of SEQ ID NO: 2; and wherein the claimed variants of SEQ ID NO: 2 form inclusion bodies when expressed in *E.coli* and are immunogenic.

***Claim Rejections - 35 USC § 112***

9. Claims 8 and 17 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term "about one third of the C-terminal of the SpaA protein is deleted" in the claims is a relative term which renders the claim indefinite. The term "about one third" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The “about one third” is “vague and indefinite” because the upper and lower limits of “about one third is not defined by the specification and one of skill in the art would not know the

metes and bounds of the invention as claimed. Applicants can obviate this rejection by stating which residues of the SpaA protein are deleted.

***Status of Claims***

Claims 8 and 17 are rejected. Claims 1-7 and 26-45 are withdrawn. No claims allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to OLUWATOSIN OGUNBIYI whose telephone number is (571)272-9939. The examiner can normally be reached on M-F 5:30 am- 2:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Oluwatosin Ogunbiyi/

Primary Examiner, Art Unit 1645